Worms that repair: reconstruction of tubes is faster than normal growth in *Spirobranchus akitsushima* (Annelida: Serpulidae)

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Abstract

Marine worms of the family Serpulidae (Annelida) construct calcareous tubes that adhere to hard substrata. The tubes of the *Spirobranchus kraussii* complex have a flat projection, known as a “flap,” over the opening of the tube. In the present study, we examined the reconstruction growth rates of the flap of *Spirobranchus akitsushima* after artificial removal, compared with the normal growth rates (control), for approximately a month at a study site in Shirahama, Wakayama Prefecture, Japan. Although the difference in the length of the flap on day 8 compared with the original length (before the removal treatment) was significant in the treatment group, the differences became nonsignificant after day 13. The flap growth rate was found to be higher than the growth rates of the tubes of other serpulid species, including *Spirobranchus* sp. 5 sensu Kobayashi & Goto (2021) of the *S. kraussii* complex, as reported in previous studies. In contrast, the length of the flap in the control group did not differ significantly during the survey period. These results indicate that the flap is more quickly reconstructed after removal compared with growth under normal conditions. Our findings imply that the flap may be of importance for the survival of *S. akitsushima*.

Keywords: annelids, biofouling, intertidal zone, polychaetes, tubeworms

Introduction

The phylum Annelida is an ecologically diverse group of worms that contains more than 20,000 described species (Capa & Hutchings 2021). Annelids include some polychaete families that construct tubes to protect them from predators and environmental stresses. Such tube-dwelling worms, especially those in aggregation, also create microhabitats for other benthic organisms (Haines & Maurer, 1980; Zühlke et al., 1998) facilitating the settlement of sessile organisms and planktonic larvae of macrobenthos by constructing hard substrates (Gallagher et al., 1983; Thomsen & McGlathery, 2005). Usually, tube-dwelling polychaetes construct their tubes by agglutinating external particles, such as silts and shells. Serpulidae Rafinesque, 1815 is a unique family in making de novo calcareous tubes by the mixture of calcium carbonate secreted from glands at the collar and organic matrix (Hedley 1956; Neff 1971). Some serpulids, e.g., *Hydroides* Gunnerus, 1768, *Ficopomatus* Southern, 1921, and *Spirobranchus* Blainville, 1818, are well known for their aggregation on artificial marine structures, e.g., ship hulls, harbor structures, and aquaculture farms (e.g., Rouse 2000; Lewis et al., 2006; Kupriyanova et al., 2020). Such biofouling diminishes the value of these structures and leads to economic losses due to the cost of cleaning these structures. Therefore, a better understanding of the growth rate and pattern of the tubes is important from the viewpoint of fisheries science as well as maritime engineering.

The shape and sculpture of the serpulid tubes are quite diverse: e.g., tusk-shaped in *Ditrupa* Berkeley, 1835; coiled in several genera and the members of the subfamily Spirorbinae Chamberlin, 1919; with transverse ridges in some species of *Pseudochitinopoma* Zibrowius, 1969; with distinct tube peristomes in *Ficopomatus enigmaticus*; and with spines in *Janita fimbriata* (Delle Chiaje, 1822) and *Placostegus* Philippi, 1844 (see reviews by ten Hove & Kupriyanova, 2009; Ippolitov et al., 2014; Vinn et al., 2023). The species of the

Spirobranchus kraussii complex construct a conspicuous "flap," a flat projection of a keel over the mouth of the tubes (e.g., Sun et al., 2012; Simon et al., 2019) (Fig. 1B). Although the gradual increase in the tube length of the *S. kraussii* complex has been reported (Nishi, 1993; Riedi & Smith, 2015), the growth of the flap remains poorly understood.

In this study, we measured the increment in the length of the flap of *Spirobranchus akitsushima* Nishi, Abe, Tanaka, Jimi & Kupriyanova, 2022 at Shirahama, Wakayama, Japan in two experimental groups: (1) flap artificially removed and (2) control, no treatment, to obtain information on the growth rate of the flap. A Japanese species of the *S. kraussii* complex, previously treated as *S. kraussii* (Baird, 1864), has been suggested to be an undescribed species based on molecular phylogenetic analyses using specimens collected at Manazuru and Shirahama on Honshu Island (Simon et al., 2019; Kobayashi & Goto, 2021). It was recently named *S. akitsushima* (Nishi et al., 2022) (Fig. 1A).

Materials and Methods

The experiment was conducted at the intertidal rocky shore of Shirahama, Wakayama, Japan (33°41'38"N, 135°20'17"E) (Fig. 2). Specimens of *Spirobranchus akitsushima* sequenced in Kobayashi & Goto (2021) were collected from this locality. The length of the flaps during the study period was measured using untreated specimens (control, n = 13) and specimens whose flaps were removed (treatment, n = 11). Because the daily increment in the length of the flap very slight, the length was measured intermittently four times during the survey period in July and August 2020 (33 days; Period 1: days 1–8, Period 2: days 9–13, Period 3: days 14–23, and Period 4: days 24–33). The flaps at the opening of the tubes were removed using tweezers (Fig. 1C). The length of each flap, from the stem to the tip of the flap, was measured to two decimal places with a digital caliper (CD-15AX, Mitutoyo

Corporation, Japan) (measurement site indicated by a two-way arrow in Fig. 1B). The Steel-Dwass test was used for multiple comparisons of the differences in the lengths of flaps among study dates and the increment in the length of the flaps among periods. All statistical analyses were computed using R ver. 4.2.2 (R Core Team 2021).

Results and Discussion

In the control group, the length of the flap of *Spirobranchus akitsushima* changed slightly (day 8: 1.38–2.72 mm, mean = 2.24 mm, SD = 0.47; day 13: 0.84–2.82 mm, mean = 2.07 mm, SD = 0.53; day 23: 1.30–2.84 mm, mean = 2.02 mm, SD = 0.43; day 33: 1.22–2.77 mm, mean = 1.92 mm, SD = 0.43) in comparison to the original length (1.45–2.69 mm, mean = 2.24, SD = 0.45) but did not differ significantly ($P \geq 0.52$) (Fig. 3A). In contrast, in the treatment group, the length of the flaps before removal (1.48–2.31 mm, mean = 1.91 mm, SD = 0.31) was significantly longer than the length recorded on day 8 (1.29–1.82 mm, mean = 1.52 mm, SD = 0.17; $P = 0.04$); however, the measured values were not significantly different between those of the original and day 13 (1.30–2.22 mm, mean = 1.69, SD = 0.27; $P = 0.51$), day 23 (0.89–2.46 mm, mean = 1.83 mm, SD = 0.45; $P = 0.98$), or day 33 (0.71–2.48 mm, mean = 1.78, SD = 0.49; $P = 0.98$) (Fig. 3A).

In each survey period, the daily increment in the length of the flap did not significantly vary in the control group (mean = −0.01 mm/day in Periods 1, 2, and 4; SD = 0.05, 0.08, and 0.01, respectively; mean = 0.00 mm/day in Period 3; SD = 0.05; the negative value is probably due to measurement errors) ($P \geq 0.91$). The daily increment in the treatment group was significantly larger in Period 1 than in the other periods ($P < 0.001$), although there were no significant differences in the measurements recorded in Periods 2–4 ($P \geq 0.29$) (Fig. 3B). In comparing the increments of the control and treatment groups in the same periods, the differences were not significant ($P \geq 0.19$), with the exception of Period 1. During Period 1, the increment in the treatment group (0.16–0.23 mm/day, mean = 0.19 mm/day, SD = 0.02) was larger than that in the control group (−0.14–0.08 mm/day, mean = −0.01 mm/day, SD = 0.05; $P < 0.001$).

Our results indicated that the length of the flaps changed slightly in the control group, i.e., under normal conditions. The flaps were, however, reconstructed and reached a length similar to the length before treatment in 13 days; this rate of growth was much greater than that under normal conditions. Because the tubes are essential for survival, the worms may have allocate additional energy to build their tubes when damaged. In a previous study, transverse mineralized tabulae, which are transverse internal tube elements used for a response to tube damage (see ten Hove & Kupriyanova, 2009), in *Serpula israelitica* Amoureux, 1977 are suggested to be a structure that closes the broken posterior end of its tube and the tabulae might be important for the species not to sink into the soft bottom (Sanfilippo, 2009). It has been suggested that the tubes of annelids protect the organism from various stresses, such intertidal waves (Stewart et al., 2004), which supports the idea that repairing damaged tubes may be important for their survival.

The incremental growth of the flaps of *S. akitsushima*—0.19 mm/day during Period 1 (Fig. 3B) in the treatment group—is also somewhat greater than the growth rate of tubes in other serpulid species examined in the previous studies; the growth rate of the tubes of several species of the *Spirobranchus kraussii* complex, to which *S. akitsushima* belongs (Simon et al., 2019; Pazoki et al., 2020; Kobayashi & Goto, 2021; Nishi et al., 2022), has been analyzed (Crisp, 1977; Nishi, 1993; Riedi & Smith, 2015). The mean growth rate of the tubes of the three species of the complex is estimated as follows: 0.046 mm/day for *Spirobranchus cariniferus* (Gray, 1843) in New Zealand (calculated from mean annual tube growth results of 1.7 cm) (Riedi & Smith, 2015); 0.087 mm/day for *Spirobranchus* sp. 5

sensu Kobayashi & Goto (2021) on Okinawa Island, Japan (as *S. kraussii* in Nishi, 1993; page 34 & fig. 8, not 0.875 mm/day, as stated in the Abstract); ≤ 0.13 mm/day for *Spirobranchus sinuspersicus* Pazoki, Rahimian, Struck, Katouzian & Kupriyanova, 2020 in Kuwait (as *Pomatoleios kraussii*; Crisp, 1977). Although the correlation between tube growth and latitudes has been suggested (slower in higher latitudes) except for *S. cariniferus* (Riedi & Smith 2015), the growth rate of the flap of *S. akitsushima* is also faster than the tube growth of species of *Spirobranchus* in lower latitudes (Okinawa Island and Kuwait). The growth rate of another serpulid species, *Galeolaria hystrix* Mörch, 1863, is estimated to be 0.11 mm/day (calculated from the mean annual tube growth of 4.0 cm) (Riedi & Smith, 2015), which is also slower than the results of the present study. These comparisons consistently indicate that the growth rate of the flap observed in the present study is faster than the tube growth of serpulids.

It may be of interest to examine the functions of the flaps of *S. akitsushima* and its relatives, given that these organisms inhabit intertidal zones that are subject to severe fluctuations in environmental factors, such as waves, temperatures, and desiccation during low tide. Although the flap might provide a sunshade for the worms to avoid ultraviolet light and heat when they spread their radioles with their radioles out of the tubes during high tide, the direction of the tube openings differs among individuals (Fig.2C). The flap is the structure around the tube opening and it might affect the movement of seawater around the tube opening and/or might support the positioning of a peduncle, at least the flap seems not to interfere with the underwater movement of radioles of *S. akitsushima* in the field observation. More field experiments that focus on examining the functions of the flap would be needed to understand the function of the flap for the survival of the species in the future.

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Figures

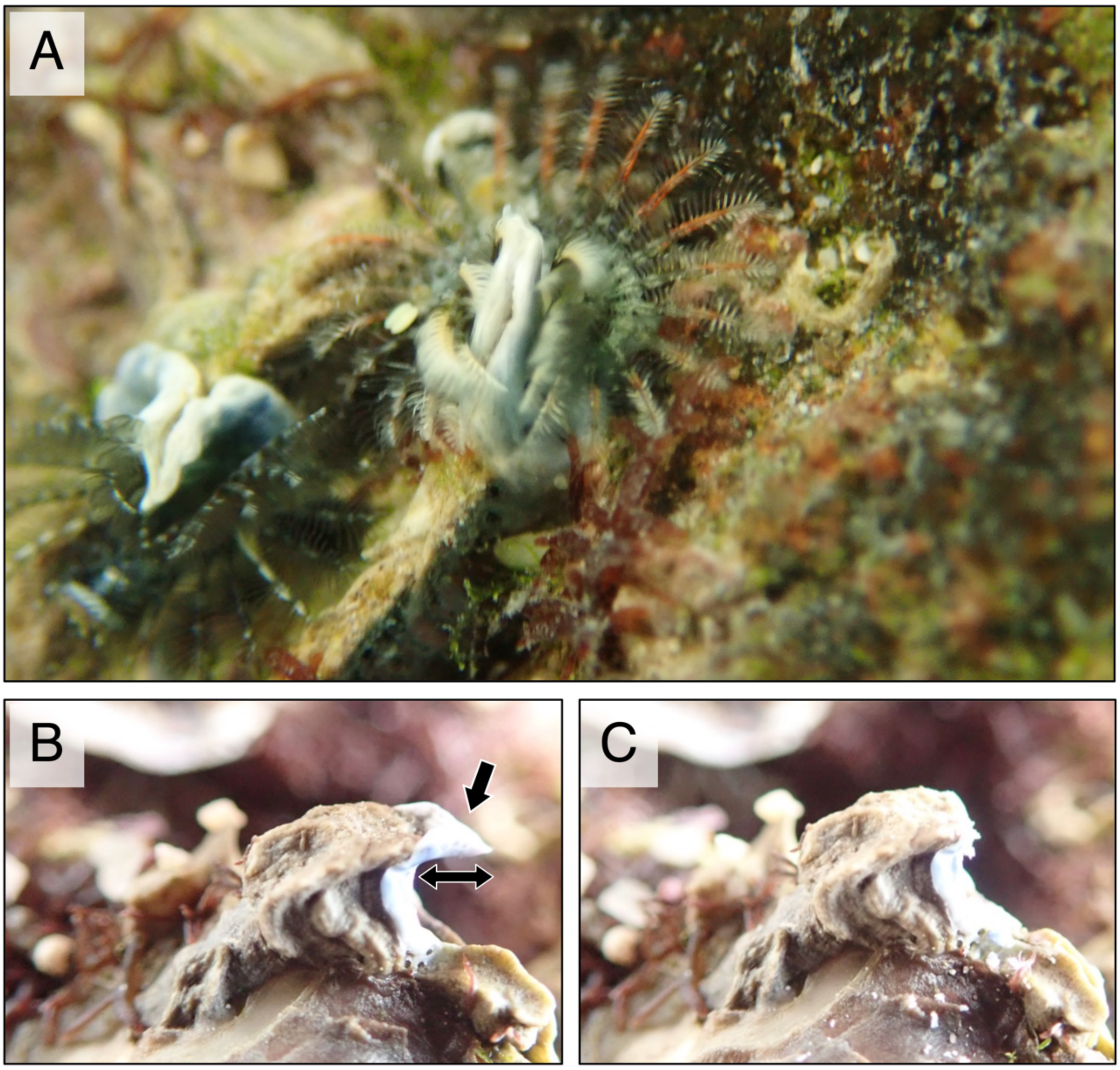

Figure 1. Photographs of *Spirobranchus akitsushima*. A: Underwater photograph of living individuals in the field in Shirahama, Wakayama Prefecture, Japan. B: Opening of the tube before treatment, lateral view, photographed during low tide. An arrow indicates the flap. A two-way arrow represents the position for measurements. C: tube after removal of the flap (the same individual as in B).

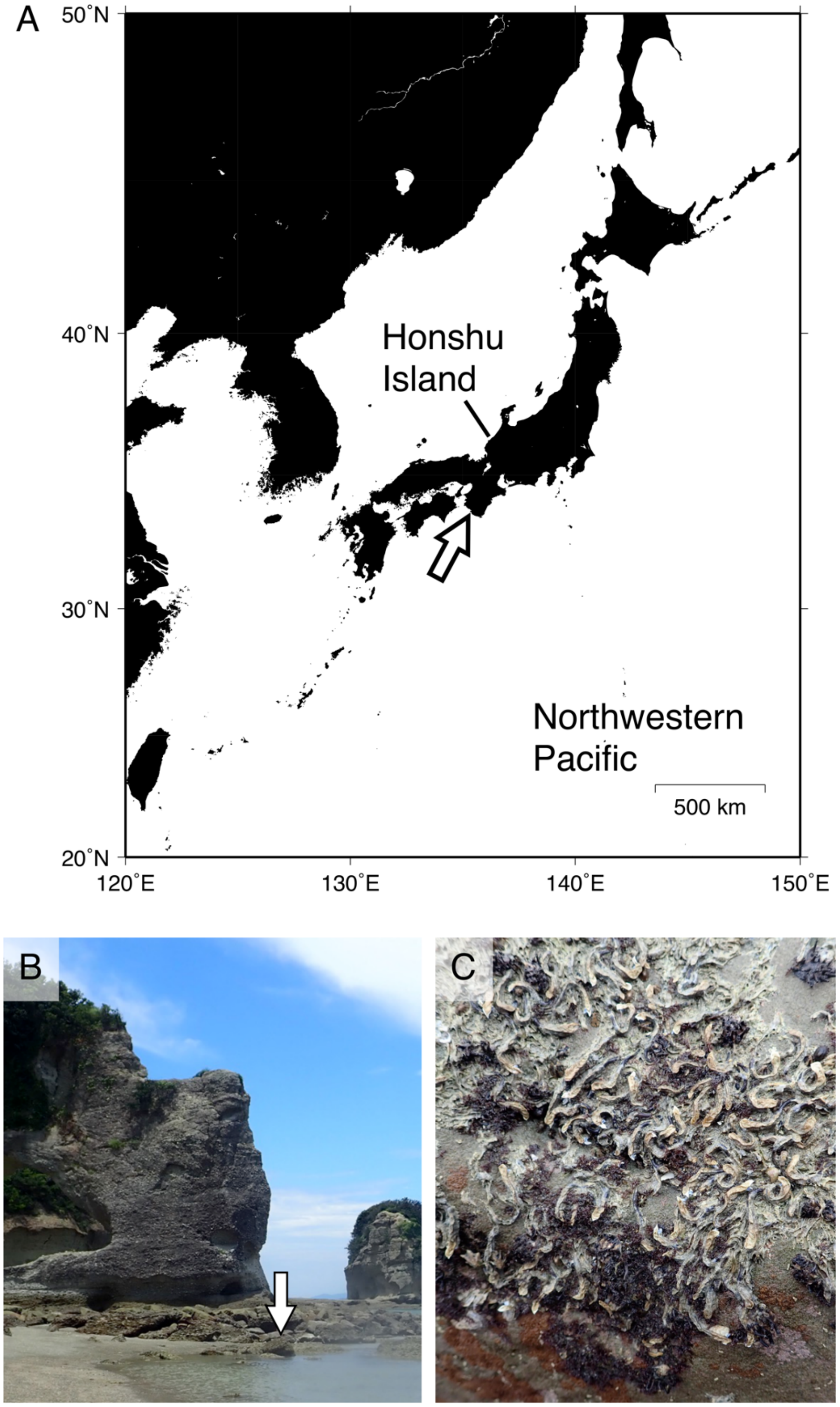

Figure 2. The study site at Shirahama, Wakayama, Japan. A: Locality of the study site (indicated by an arrow). B: Landscape of the study site. An arrow indicates the location of the rock shown in C. C: Aggregated tubes of *Spirobranchus akitsushima* on the rock during low tide.

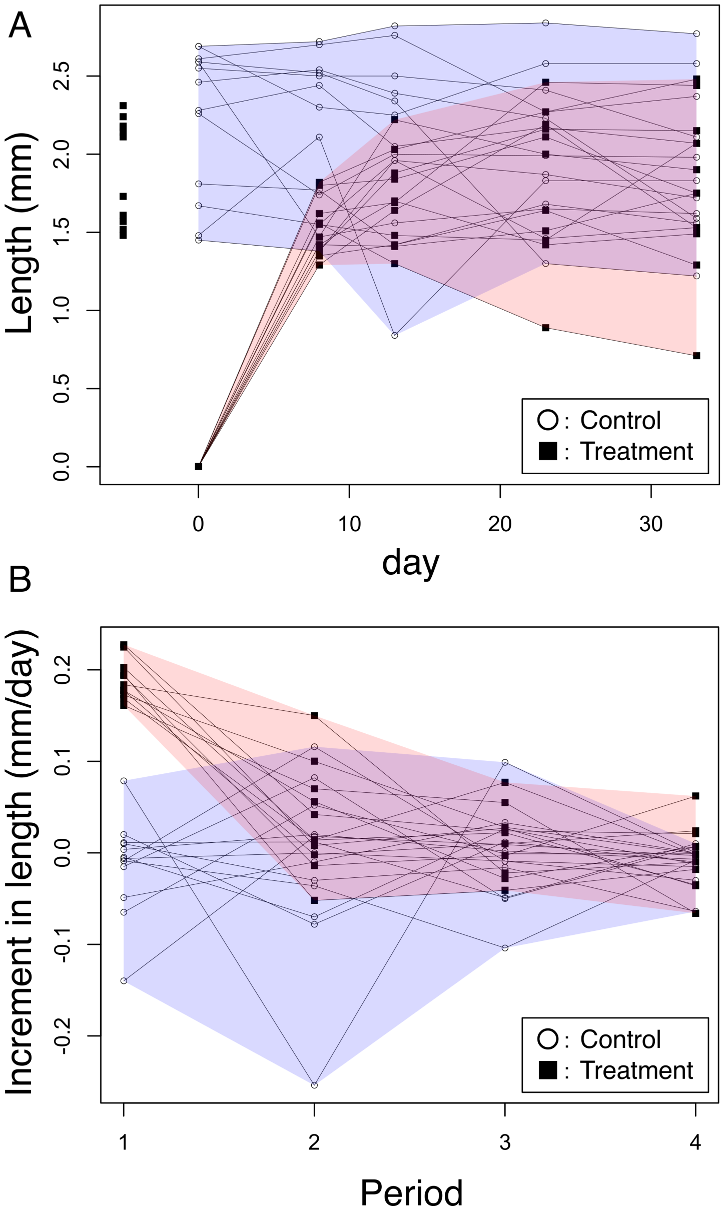

Figure 3. Growth of the flap of *Spirobranchus akitsushima*. Open circles and closed squares indicate untreated specimens (control) and specimens whose flaps were removed (treatment), respectively. Blue and red highlights show the ranges of the flap length of the control and treated specimens, respectively. A: Length of the flap during the survey period. Squares plotted on the left side of day 0 represent the length of flaps of the treated specimens before the treatment (original lengths). B: Daily increment in flap length in each period of measurement. Period 1: days 1–8, Period 2: days 9–13, Period 3: days 14–23, and Period 4: days 24–33.